Published in final edited form as:

J Magn Reson. 2017 February ; 275: 120–126. doi:10.1016/j.jmr.2016.12.009.

Hyperpolarized ^{13}C pyruvate mouse brain metabolism with absorptive-mode EPSI at 1 T

Vesselin Z. Miloushev^{a,*}, Valentina Di Gialleonardo^{a,b}, Lucia Salamanca-Cardona^{a,b}, Fabian Correa^{a,b}, Kristin L. Granlund^{a,b}, and Kayvan R. Keshari^{a,b,c,*}

^aDepartment of Radiology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

^bMolecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

^cWeill Cornell Medical College, New York, NY 10065, USA

Abstract

The expected signal in echo-planar spectroscopic imaging experiments was explicitly modeled jointly in spatial and spectral dimensions. Using this as a basis, absorptive-mode type detection can be achieved by appropriate choice of spectral delays and post-processing techniques. We discuss the effects of gradient imperfections and demonstrate the implementation of this sequence at low field (1.05 T), with application to hyperpolarized [$1\text{-}^{13}\text{C}$] pyruvate imaging of the mouse brain. The sequence achieves sufficient signal-to-noise to monitor the conversion of hyperpolarized [$1\text{-}^{13}\text{C}$] pyruvate to lactate in the mouse brain. Hyperpolarized pyruvate imaging of mouse brain metabolism using an absorptive-mode EPSI sequence can be applied to more sophisticated murine disease and treatment models. The simple modifications presented in this work, which permit absorptive-mode detection, are directly translatable to human clinical imaging and generate improved absorptive-mode spectra without the need for refocusing pulses.

Keywords

Hyperpolarized MRI; [$1\text{-}^{13}\text{C}$] pyruvate; Mouse brain; Absorptive mode; EPSI

1. Introduction

Hyperpolarized MRI (HP MRI) is well suited to imaging *in vivo* metabolism by dramatically amplifying the MR signal from small molecules central to endogenous metabolic processes. The most widespread hyperpolarization method, dissolution dynamic nuclear polarization (DNP) can increase the signal of small metabolites approximately 10,000 fold, enabling detection of metabolites at micromolar to nanomolar concentrations [1,2]. *In vivo* imaging can be achieved by intravenous administration of HP molecules,

*Corresponding authors at: Department of Radiology, Schwartz Building S121E, 1275 York Avenue, New York, NY 10065, USA (V.Z. Miloushev). Department of Radiology and Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA (K.R. Keshari). miloushv@mskcc.org (V.Z. Miloushev), rahimikk@mskcc.org (K.R. Keshari).

similar to traditional contrast agents, although with emergent properties as metabolic biomarkers [3].

Pyruvate is emerging as a canonical HP probe for central energy metabolism, due to its ability to distinguish reductive from oxidative central energy metabolism and the favorable polarization and relaxation properties of $[1-^{13}\text{C}]$ pyruvate [4]. The conversion of hyperpolarized $[1-^{13}\text{C}]$ pyruvate to lactate (reductive metabolism, flux through lactate dehydrogenase) and bicarbonate (oxidative metabolism, flux through pyruvate dehydrogenase) has been demonstrated in the anesthetized rat brain and a non-human primate [5,6]. In the anesthetized rat brain, hyperpolarized pyruvate is preferentially metabolized to lactate, with bicarbonate detected at low levels [7]. Applications to pre-clinical glioma models have demonstrated increased lactate production in tumors compared to the adjacent brain, and modification of lactate production with treatment [5,8–10].

Extending this work to mouse brain tumor models permits application to a wider array of pre-clinical models and the use of newly synthesized hyperpolarized probes, as recently demonstrated [11–13]. Imaging the smaller mouse brain however presents unique challenges. Modern hyperpolarized spectroscopic imaging approaches commonly use echo-planar spectroscopic imaging (EPSI) acquisitions, a chemical shift imaging technique similar to echo-planar imaging, in which phase encoding is replaced by chemical shift evolution between successive frequency encoding gradients [14–17]. Recent advances to this method include a fly-back design, double spin-echo refocusing, and sparse sampling [18–21].

In this work, an absorptive-mode EPSI sequence is developed using a full-dwell initial spectral delay [22]. Absorptive mode lineshapes result in improved spectral separation compared to magnitude mode lineshapes [23]. The absorptive mode sequence is implemented at low field strength (1.05 T), similar to the predominant field strengths used for clinical imaging (1.5–3 T). Hyperpolarized spins in many molecules of interest have longer lifetimes at lower field strengths due to the field dependence of the T_1 time constant, ultimately resulting in an increased signal-to-noise ratio (SNR). However, spectral resolution is inherently decreased at lower field strengths, motivating development of an absorptive-mode sequence. We demonstrate the application of this sequence to hyperpolarized pyruvate metabolism in the normal mouse brain. The simple modifications presented in this work are directly translatable to human clinical imaging.

2. Materials and methods

2.1. Imaging and spectroscopy

Spectroscopic imaging was performed on a small animal imaging system (Nanoscan Mediso Inc.) with a permanent magnet operating at a static magnetic field of 1.05 T. The drift of this magnet system is variable, but observed to be up to 1 ppm per minute for ^1H ; this does not result in significant line broadening in EPSI experiments, which are rapidly acquired. Moreover, a simple ^1H experiment can be used for center frequency calibration, just prior to the ^{13}C experiment. All imaging experiments were performed with a dual tuned ^1H - ^{13}C coil. An absorptive-mode, fly-back EPSI sequence was used (Fig. 1A). For all EPSI sequences,

the initial spectral delay was set to a full-dwell. This delay included time for the excitation pulse and the slice-selective, unwinding, and phase encoding gradients. The gradient ramp time was 250 μ s and the gradient plateau time prior to detection was 125 μ s. To account for unwinding and fly-back gradient errors, gradients were calibrated with ^1H phantoms (with appropriate scaling for the gyromagnetic ratio).

For phantom experiments, the 2D absorptive-mode fly-back EPSI was compared to a 2D CSI sequence, both acquired with a full-dwell initial delay (see Fig. 3). Phantoms contained 7 M ^{13}C Urea in glycerol or 4 M ^{13}C acetate, 4 M ^{13}C Urea, and 1 M $1\text{-}^{13}\text{C}$ Alanine with 1 mM Gadodiamide. The EPSI spectral width was 19 ppm with 2 mm spatial resolution. The EPSI phase encode dimension was 17 points, acquired as an interleaved echo (sampling the zero-center of k-space first), with 17 excitations (nex) per point. For the CSI sequence the matrix size was 17×17 with 1 nex, and a center-out Cartesian spiral trajectory was used to sample k-space.

For hyperpolarized animal experiments, 1D or 2D absorptive-mode fly-back EPSI type sequences were used. A slice-selective 15° (1D) or 30° (2D), 600–1200 μ s 8-lobe sinc pulse depending on the necessary EPSI spectral sweep width, and 10 mm slice thickness, calibrated based on ^1H signal just prior to the experiment. The dynamic 1D EPSI was acquired with a spectral sweep-width of 32.3 ppm (363 Hz, 1.6 ms spatial acquisition time (τ_1) and 1.15 ms gradient time (τ_g)), and 2 mm EPSI spatial resolution. The 2D EPSI was acquired with a spectral sweep-width of 19 ppm, (212 Hz, 3.2 ms spatial acquisition time (τ_1) and 1.5 ms gradient time (τ_g)), 4 mm spatial resolution. The indirectly detected phase-encoded dimension was acquired as an interleaved echo (9 points, sampling the zero-center of k-space first) with 3.5 mm resolution. Anatomic imaging was performed using a T_2 -weighted sequence, with an axial plane resolution of 0.25×0.25 mm and 2 mm slice thickness.

Quantification of polarization was performed on a 1.0 T permanent magnet bench-top NMR spectrometer equipped with a dual tuned ^1H - ^{13}C coil (Magritek Ltd.). Sequential pulse-and-acquire experiments with a 5-degree flip angle and 5 s repetition time were used to measure the polarization decay. The apparent T_1 time constant was estimated from the signal decay, correcting for the excitation flip angle. A thermal equilibrium spectrum was acquired with a 30-degree flip angle, 10 s repetition time (TR), and 256 scans (see Supplementary Materials). The polarization level was estimated by comparing the dynamic spectra and the thermal equilibrium spectrum and correcting for flip angle, number of signal averages, and T_1 decay.

The flip angle-corrected HP [$1\text{-}^{13}\text{C}$] pyruvate $T_1 = 70.8 \pm 0.7$ s at 1.0 T was used to approximate T_1 losses between dissolution and imaging (45 s). The *in vivo* HP pyruvate concentration was estimated from polarization enhancement and comparing peak integrals with a 5 M ^{13}C urea phantom (with 1 mM Gd-DOTA), with corrections for number of scans, and for the flip angle.

2.2. Animals

This study was performed in accordance with an Institutional Animal Care Use Committee (IACUC)-approved protocol (MSKCC #13-12-019, PI: Keshari) for hyperpolarized magnetic resonance imaging of mice. BALB/c mice were obtained from Jackson Laboratories. Anesthesia was performed using continuous flow of 1–2% isoflurane. Injection of hyperpolarized pyruvate was performed via tail vein cannulation. A total of 6 mice were used for imaging, 3 for 1D studies and 3 for 2D studies.

2.3. Hyperpolarized probes

Dissolution dynamic nuclear polarization (DNP) was performed with a SpinLab Hyperpolarizer (General Electric) operating at 3.35 T. The sample mixture (100 μ L) was composed of 14.2 M [1- 13 C] pyruvic acid and 15 mM trityl radical (General Electric) [24]. Spin polarizations of approximately 20% were achieved in 2 h. The samples were dissolved with 11 mL of buffer (40 mM TRIS at pH 7.4, 1 mM EDTA) into a pre-cooled vessel. The pH of the dissolute was titrated to physiological range by stoichiometric addition of NaOH. Animal injection was initiated approximately 20–30 s following dissolution, and 200 μ L were injected over approximately 10 s. Dynamic 1D EPSI imaging was initiated at the start of the injection. 2D EPSI was initiated 15 s after the end of injection to allow time for lactate conversion.

2.4. Processing

Data processing was performed using standard MATLABTM (The Mathworks Inc., Natick, MA) functions. The spectral dimension was apodized using a Kaiser window ($\beta = 2$). The EPSI spectral dimension was echo-symmetrized (zero-filled to twice the size) prior to Fourier transformation, and spatial dimensions were zero-filled to twice the size [25,26]. The 360° first order phase shift in the spectral dimension due to full dwell delay was accomplished by equivalently zero-padding the spectral time dimension with a single time point, prior to Fourier transformation. Because spectra were sparse, baseline offset correction could be performed using a histogram method (the baseline being the most common value and adjusting appropriately).

Spectrally dependent shifts in the EPSI spatial dimension were corrected in the frequency dimension. Fine interpolation (5 $\times$) was used prior to shifting to account for fractional shifts on the order of 0.2 $\times$ voxel size. Please note that this method cannot adequately correct aliased resonances without prior knowledge.

The residual unwinding gradient error, which is approximately a small time shift, equivalent to a first-order phase shift, was corrected by maximum entropy minimization on the first derivative of the resulting Fourier transformed image spectra [27]. Global minima were first found using a coarse grid search and subsequently refined. No post-processing adjustment was made for accumulation of the fly-back gradient error, as these gradients were calibrated on ^1H phantoms.

For the dynamic 1D EPSI experiment, the dynamic time course curves represent spectral integrals within 1 ppm of the resonance maximum, with 2-fold spline interpolation. Average

($n = 3$) maximum time-course pyruvate and lactate curves are reported with standard errors in the mean, without correction for flip angle. The lactate metabolism curves were averaged after aligning the pyruvate delivery curves. For static 2D experiments, metabolic maps represent spectral integral within 1 ppm of the resonance maximum.

3. Results

3.1. Theory: Absorptive mode fly-back EPSI detection

The phase-sensitive NMR signal from an object located between spatial angular frequencies ω_a and ω_b along a gradient direction is modeled as:

$$S_{obj}(t) = \int_{\omega_a}^{\omega_b} \exp(i\omega t - 1/T_2^* t) \partial\omega = i \exp(-1/T_2^* t) \frac{\exp(i\omega_a t) - \exp(i\omega_b t)}{t}. \quad (1)$$

with $\lim_{t \rightarrow 0} S_{obj} = \omega_b - \omega_a$.

If the signal is acquired as an echo with an effective refocusing period TE:

$$S_{obj}(t) = i \exp(-1/T_2^* t) \frac{\exp(i\omega_a(t - TE)) - \exp(i\omega_b(t - TE))}{t - TE}. \quad (2)$$

The signal from a fly-back EPSI-type sequence is modeled as:

$$S_{fbEPSI}(t_1, t_2) = S_{obj}(t_1) \cdot \exp((i\Omega - 1/T_2^*)(t_1 + t_2 + \delta)). \quad (3)$$

In the above, Ω is the spectral angular frequency, $t_1 = 0 \dots \tau_1$ is the time of the directly detected spatial (i.e., frequency) dimension, $TE = \tau_1/2$ is the refocusing time, $t_2 = 0 \dots \tau_2$ is the time of the indirectly detected spectral dimension, and δ is the initial delay prior to the start of the acquisition. Phased absorptive-mode spectra can be acquired with spectral delays of zero-dwell (0), half-dwell ($\pi/2$), or full-dwell (π) initial delays [22]; the zero-dwell and half-dwell delays result in no baseline distortion, while the full-dwell delay results in a constant baseline offset.

For $n/2$ -dwell, setting $\delta = n/2 \cdot TE$ results in:

$$S_{fbEPSI}(t_1, t_2) = S_{obj}(t_1) \cdot \exp((i\Omega - 1/T_2^*)(t_1 - TE)) \cdot \exp((i\Omega - 1/T_2^*)(t_2 + n\Delta/2)). \quad (4)$$

The component in the spatial (t_1) dimension,

$$\exp((i\Omega - 1/T_2^*)(t_1 - TE)), \quad (5)$$

is an echoed spectrum in the spatial t_1 dimension, and the spectral angular frequency Ω has a small effect on the spatial angular frequency. This spatial shift is correctable up to aliasing (see Materials and Methods, Fig. 2B).

The component in the spectral (t_2) dimension,

$$\exp((i\Omega - 1/T_2^*)(t_2 + n\Delta/2)), \quad (6)$$

is a phase-able absorptive-mode spectrum if $n = 0, 1, 2$. The resultant pulse sequence diagram is provided in Fig. 1A. The choice of appropriate initial delay, δ , effectively simplifies the k-space trajectories (Fig. 1B). Please see the Supplementary Materials for a similar derivation of symmetric EPSI signals.

The choice of half-dwell versus full-dwell detection depends on the time required for direct frequency encoding gradient ramps and fly-back gradient refocusing, τ_g , during an EPSI fly-back loop. Given a sweep width for the spectral dimension, $sweep = 1/\Delta$, where $\Delta = \tau_1 + \tau_g$, half-dwell detection requires a spectral delay,

$$\delta_{\Delta/2} = (\tau_1 + \tau_g)/2 - \tau_1/2 = \tau_g/2. \quad (7)$$

Hence, this sets a limit on the spectral delay, in which additional slice refocusing or multidimensional phase-encode gradients need to be applied to prepare magnetization prior to EPSI detection.

In general, the “efficiency” of fly-back EPSI-type sequences is maximized using a minimum sufficient sweep width (with aliasing as appropriate), so as to maximize the time for encoding τ_1 and minimize τ_g , during which no encoding occurs. Increasing τ_g to accommodate half-dwell detection will provide baseline offset correction at the expense of SNR efficiency.

In the case of full-dwell detection,

$$\delta_{\Delta} = (\tau_1 + \tau_g) - \tau_1/2 = \tau_1/2 + \tau_g, \quad (8)$$

allowing more time to prepare magnetization prior to EPSI detection at the expense of T_2^* losses.

We note that the alternative approach of time-shifting in the t_2 dimension to zero-dwell or half-dwell is possible, due to the Fourier circular time-shift property. However, the discrete Fourier transform circular time shift property is exact only for integer time shifts of periodic signals. Ringing artifacts become more pronounced when a limited number of points are acquired in the EPSI spectral dimension and there is discontinuity at the edges of the time series; this is less of an issue for echoed signals, such as in the spatial dimension. We note that these problems could be addressed by alternative methods of interpolation of the time

series or by applying additional constraints on the transformed spectrum or by directly fitting the time series. Lastly, we note that exactly phasing a spectrum with an arbitrary delay is possible provided that the signal is appropriately band-limited, which is what half-dwell detection enforces.

3.2. Simulation: Absorptive-mode fly-back EPSI and gradient errors

The proposed absorptive-mode fly-back EPSI sequence was validated by numerical simulation (Fig. 2A). The spectrally dependent spatial shift of fly-back EPSI type experiments (Eq. (5)) is small and correctable (Fig. 2B). The approach to correction in this work is to reverse the spectrally dependent spatial shift in the Fourier transform domain, after fine-interpolation. Small errors in the refocusing gradient result in a time shift, which can be corrected with a first-order phase correction in the t_1 dimension (Fig. 2C). This phase error can be determined from maximum entropy minimization of the 2D EPSI spectral-spatial spectrum. On the other hand, errors in the EPSI fly-back gradient that accrue over every gradient refocusing lobe, lead to “skew” distortions of the object in the spectral and spatial dimension (Fig. 2D). These “skew” distortions are also correctable by appropriate time-shifting/first order phasing to some extent if known a priori, which can be calibrated using phantom data. In this work, we corrected the gradient errors prior to acquisition. Finally, the absorptive-mode EPSI was implemented and tested on ^{13}C phantoms (Fig. 3).

3.3. Experimental results

Metabolic imaging of the normal mouse brain (under anesthesia) was performed with hyperpolarized pyruvate. Given the anatomy of the mouse head (relatively thin scalp soft tissue), a 1D EPSI imaging scheme was sufficient to localize spectroscopic imaging to the mouse brain (Fig. 4A). A dynamic 1D EPSI scan was used to acquire both spatial and spectral information with a temporal resolution of 3 s. Pyruvate and lactate were readily resolved in regions encompassing the brain and the vessels in the soft tissue ventral to the skull base (Fig. 4B). Time-resolved 1D EPSI imaging demonstrates the expected gamma-variate curve of pyruvate perfusion and conversion to lactate (Fig. 4C). The maximum brain lactate signal occurred 18 ± 1 s post injection, which was delayed relative to the soft tissues, in which the maximum lactate signal occurred 13 ± 3 s post injection (Fig. 4C).

Based on the dynamic imaging results and to allow sufficient lactate buildup, the static 2D EPSI imaging was acquired at 25 s post injection (Fig. 5). The 2D EPSI image was overlaid on T2-weighted image for anatomical reference (Fig. 5A). Average spectra from the mouse brain and soft tissues demonstrate higher lactate signal in the brain than the soft tissues (Fig. 5B). Bicarbonate and pyruvate hydrate were not detected in the brain or soft tissues in our experiment, probably to due to insufficient SNR. Alanine was detected in the soft tissues but not in the brain (Fig. 5B). Comparing peak integrals with a 5 M ^{13}C urea phantom and accounting for polarization and T_1 decay prior to imaging, we estimate the *in vivo* pyruvate and lactate concentrations to be on the order 200 μM or approximately 200 nmol/g in the mouse brain, which agrees with previously reported values [5]. Metabolic maps demonstrate higher lactate signal in the brain, underscoring that the brain is more metabolically active than the ventral soft tissues of the head and neck (Fig. 5CD).

4. Discussion

The majority of clinical magnetic resonance imaging systems operate at low field (1.5 T), which is favorable for hyperpolarized [$1\text{-}^{13}\text{C}$] pyruvate, due to increased lifetimes at lower field strength [4]. While higher field strengths have many well-known advantages in sensitivity and spectral resolution, lower magnetic field strengths have improved intrinsic T_1 -weighted contrast, decreased susceptibility artifacts, decreased demands on gradient performance, and may be preferred for imaging of certain patient groups (such as patients with metallic surgical hardware, clips, or devices). Absorptive-mode detection at lower field strengths is advantageous due to improved spectral separation of individual resonances. With these motivations we developed an absorptive-mode EPSI sequence and implemented it on a pre-clinical low field permanent magnet imaging system. The major disadvantages of permanent magnets are inhomogeneity and drift in time, which are not limitations for our small animal hyperpolarized study due to the fast, high-resolution acquisition.

The explicit derivation of the expected signal from EPSI-type experiments demonstrates that absorptive-mode detection is possible with an appropriate choice of the initial spectral delay. Refocusing pulses are not required in this implementation, simplifying the pulse sequence and potentially reducing the specific absorption rate (SAR) for clinical implementations. Artifacts related to spectral-spatial shifts and unwinding gradient errors can be corrected during post-processing. We note that while the double spin-echo technique allows full k-space sampling, the SNR benefit depends on T_2 . While the T_2 of small molecules in solution is relatively long in relation to typical acquisition times, the physiological T_2 (i.e., in different physiological compartments or micro-environments) may be shorter [28,29]. Furthermore, generation of high-quality refocusing pulses may not be feasible given hardware and SAR constraints in the clinical setting.

Implementing the sequence on a 1.05 T permanent magnet achieves sufficient SNR to image HP [$1\text{-}^{13}\text{C}$] pyruvate metabolism in the mouse brain. We were not able to detect bicarbonate or the pyruvate hydrate probably due to limitations in signal to noise. Our results agree with prior imaging of rat brain metabolism [5]. Further work is necessary to unravel the complex metabolic interactions of different cell types in the normal brain and different tumor types [30].

The limitations of the current study design are that it is inherently a pre-clinical study using a small number of animals as a proof of principle. For this reason, extrapolation of the metabolic parameters to humans is guarded. Further, we imaged mice under anesthesia, which is known to alter brain metabolic profiles [31,32]. The quantitative parameters in this work are thus relative and not absolute. Nevertheless, imaging of awake rodents is possible without anesthesia, holding promise for future studies [33]. Moreover, it is reasonable to explore future multi-modality studies combining HP MRI and PET to characterize novel mechanisms in murine models.

The purpose of this study was to optimize spectroscopic imaging of hyperpolarized probes on low field permanent magnet imaging system. We demonstrated the feasibility of imaging the mouse brain at this clinically relevant field strength using hyperpolarized pyruvate.

Further, we believe that the simple modifications to the existing EPSI technique are translatable and beneficial to clinical imaging of hyperpolarized [$1\text{-}^{13}\text{C}$] pyruvate metabolism in the human brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding sources

Funding sponsors had no direct role in conduct of research and/or preparation of this article. We acknowledge the following funding sources:

1. MSKCC Radiology Development Project Grant (VM).
2. NIH P30 CA008748 Cancer Center Support Grant (All).
3. NIH R00 EB014328 and R01 CA195476 (KK).
4. Center for Molecular Imaging and Nanotechnology at MSKCC (KK).
5. American Italian Cancer Foundation (VDG).
6. Geoffrey Beene Cancer Research Center Grant Award (KK).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jmr.2016.12.009>.

References

1. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K. Increase in signal-to-noise ratio of $>10,000$ times in liquid-state NMR. *Proc Natl Acad Sci USA*. 2003; 100:10158–10163. [PubMed: 12930897]
2. Golman K, Ardenkjaer-Larsen JH, Petersson JS, Mansson S, Leunbach I. Molecular imaging with endogenous substances. *Proc Natl Acad Sci USA*. 2003; 100:10435–10439. [PubMed: 12930896]
3. Keshari KR, Wilson DM. Chemistry and biochemistry of ^{13}C hyperpolarized magnetic resonance using dynamic nuclear polarization. *Chem Soc Rev*. 2014; 43:1627–1659. [PubMed: 24363044]
4. Tee SS, DiGalleonardo V, Eskandari R, Jeong S, Granlund KL, Miloushev V, Poot AJ, Truong S, Alvarez JA, Aldeborgh HN, Keshari KR. Sampling hyperpolarized molecules utilizing a 1 T permanent magnetic field. *Sci Rep*. 2016; 6:32846. [PubMed: 27597137]
5. Hurd RE, Yen YF, Mayer D, Chen A, Wilson D, Kohler S, Bok R, Vigneron D, Kurhanewicz J, Tropp J, Spielman D, Pfefferbaum A. Metabolic imaging in the anesthetized rat brain using hyperpolarized [$1\text{-}^{13}\text{C}$] pyruvate and [$1\text{-}^{13}\text{C}$] ethyl pyruvate. *Magn Reson Med*. 2010; 63:1137–1143. [PubMed: 20432284]
6. Park I, Larson PE, Tropp JL, Carvajal L, Reed G, Bok R, Robb F, Bringas J, Kells A, Pivrotto P, Bankiewicz K, Vigneron DB, Nelson SJ. Dynamic hyperpolarized carbon-13 MR metabolic imaging of nonhuman primate brain. *Magn Reson Med*. 2014; 71:19–25. [PubMed: 24346964]
7. Mayer D, Yen YF, Takahashi A, Josan S, Tropp J, Rutt BK, Hurd RE, Spielman DM, Pfefferbaum A. Dynamic and high-resolution metabolic imaging of hyperpolarized [$1\text{-}^{13}\text{C}$]-pyruvate in the rat brain using a high-performance gradient insert. *Magn Reson Med*. 2011; 65:1228–1233. [PubMed: 21500253]

8. Marjanska M, Iltis I, Shestov AA, Deelchand DK, Nelson C, Ugurbil K, Henry PG. In vivo ^{13}C spectroscopy in the rat brain using hyperpolarized [1-(^{13}C) pyruvate and [2-(^{13}C)pyruvate. *J Magn Reson.* 2010; 206:210–218. [PubMed: 20685141]
9. Park I, Larson PE, Zierhut ML, Hu S, Bok R, Ozawa T, Kurhanewicz J, Vigneron DB, Vandenberg SR, James CD, Nelson SJ. Hyperpolarized ^{13}C magnetic resonance metabolic imaging: application to brain tumors. *Neuro Oncol.* 2010; 12:133–144. [PubMed: 20150380]
10. Park JM, Recht LD, Josan S, Merchant M, Jang T, Yen YF, Hurd RE, Spielman DM, Mayer D. Metabolic response of glioma to dichloroacetate measured in vivo by hyperpolarized (^{13}C) magnetic resonance spectroscopic imaging. *Neuro Oncol.* 2013; 15:433–441. [PubMed: 23328814]
11. Radoul M, Chaumeil MM, Eriksson P, Wang AS, Phillips JJ, Ronen SM. MR studies of glioblastoma models treated with dual PI3K/mTOR inhibitor and temozolomide: metabolic changes are associated with enhanced survival. *Mol Cancer Ther.* 2016; 15:1113–1122. [PubMed: 26883274]
12. Chen Y, Kim H, Bok R, Sukumar S, Mu X, Sheldon RA, Barkovich AJ, Ferriero DM, Xu D. Pyruvate to lactate metabolic changes during neurodevelopment measured dynamically using hyperpolarized ^{13}C imaging in juvenile murine brain. *Dev Neurosci.* 2016; 38:34–40. [PubMed: 26550989]
13. Eichhorn TR, Takado Y, Salameh N, Capozzi A, Cheng T, Hyacinthe JN, Mishkovsky M, Roussel C, Comment A. Hyperpolarization without persistent radicals for in vivo real-time metabolic imaging. *Proc Natl Acad Sci USA.* 2013; 110:18064–18069. [PubMed: 24145405]
14. Brown TR, Kincaid BM, Ugurbil K. NMR chemical shift imaging in three dimensions. *Proc Natl Acad Sci USA.* 1982; 79:3523–3526. [PubMed: 6954498]
15. Guilfoyle DN, Mansfield P. Chemical-shift imaging. *Magn Reson Med.* 1985; 2:479–489. [PubMed: 4094561]
16. Posse S, DeCarli C, Le Bihan D. Three-dimensional echo-planar MR spectroscopic imaging at short echo times in the human brain. *Radiology.* 1994; 192:733–738. [PubMed: 8058941]
17. Mulkern RV, Panych LP. Echo planar spectroscopic imaging. *Concepts Magn Reson.* 2001; 13:213–237.
18. Cunningham CH, Vigneron DB, Chen AP, Xu D, Nelson SJ, Hurd RE, Kelley DA, Pauly JM. Design of flyback echo-planar readout gradients for magnetic resonance spectroscopic imaging. *Magn Reson Med.* 2005; 54:1286–1289. [PubMed: 16187273]
19. Cunningham CH, Chen AP, Albers MJ, Kurhanewicz J, Hurd RE, Yen YF, Pauly JM, Nelson SJ, Vigneron DB. Double spin-echo sequence for rapid spectroscopic imaging of hyperpolarized ^{13}C . *J Magn Reson.* 2007; 187:357–362. [PubMed: 17562376]
20. Yen YF, Kohler SJ, Chen AP, Tropp J, Bok R, Wolber J, Albers MJ, Gram KA, Zierhut ML, Park I, Zhang V, Hu S, Nelson SJ, Vigneron DB, Kurhanewicz J, Dirven HA, Hurd RE. Imaging considerations for in vivo ^{13}C metabolic mapping using hyperpolarized ^{13}C -pyruvate. *Magn Reson Med.* 2009; 62:1–10. [PubMed: 19319902]
21. Larson PEZ, Hu S, Lustig M, Kerr AB, Nelson SJ, Kurhanewicz J, Pauly JM, Vigneron DB. Fast dynamic 3D MRSI with compressed sensing and multiband excitation pulses for hyperpolarized (^{13}C) studies. *Magn Reson Med.* 2011; 65:610–619. [PubMed: 20939089]
22. Zhu G, Torchia DA, Bax A. Discrete Fourier transformation of NMR signals. The relationship between sampling delay time and spectral baseline. *J Magn Reson Ser A.* 1993; 105:219–222.
23. Ernst, RR., Bodenhausen, G., Wokaun, A. Principles of Nuclear Magnetic Resonance in One and Two Dimensions. Clarendon Press; Oxford: 1987.
24. Chen AP, Albers MJ, Cunningham CH, Kohler SJ, Yen YF, Hurd RE, Tropp J, Bok R, Pauly JM, Nelson SJ, Kurhanewicz J, Vigneron DB. Hyperpolarized C-13 spectroscopic imaging of the TRAMP mouse at 3T-initial experience. *Magn Reson Med.* 2007; 58:1099–1106. [PubMed: 17969006]
25. Bartholdi E, Ernst RR. Fourier spectroscopy and the causality principle. *J Magn Reson.* 1973; 11:9–19.

26. Ebel A, Dreher W, Leibfritz D. Effects of zero-filling and apodization on spectral integrals in discrete Fourier-transform spectroscopy of noisy data. *J Magn Reson.* 2006; 182:330–338. [PubMed: 16844391]
27. Chen L, Weng Z, Goh L, Garland M. An efficient algorithm for automatic phase correction of NMR spectra based on entropy minimization. *J Magn Reson.* 2002; 158:164–168.
28. Reed GD, von Morze C, Bok R, Koelsch BL, Van Crieginge M, Smith KJ, Shang H, Larson PEZ, Kurhanewicz J, Vigneron DB. High resolution (13)C MRI with hyperpolarized urea. In vivo T(2) mapping and (15)N labeling effects. *IEEE Trans Med Imag.* 2014; 33:362–371.
29. Yen YF, Le Roux P, Mayer D, King R, Spielman D, Tropp J, Butts Pauly K, Pfefferbaum A, Vasanawala S, Hurd R. T(2) relaxation times of (13)C metabolites in a rat hepatocellular carcinoma model measured in vivo using (13)C-MRS of hyperpolarized [1-(13)C]pyruvate. *NMR Biomed.* 2010; 23:414–423. [PubMed: 20175135]
30. Turner DA, Adamson DC. Neuronal-astrocyte metabolic interactions: understanding the transition into abnormal astrocytoma metabolism. *J Neuropathol Exp Neurol.* 2011; 70:167–176. [PubMed: 21293295]
31. Du F, Zhang Y, Iltis I, Marjanska M, Zhu XH, Henry PG, Chen W. In vivo proton MRS to quantify anesthetic effects of pentobarbital on cerebral metabolism and brain activity in rat. *Magn Reson Med.* 2009; 62:1385–1393. [PubMed: 19780161]
32. Makaryus R, Lee H, Yu M, Zhang S, Smith SD, Rebecchi M, Glass PS, Benveniste H. The metabolomic profile during isoflurane anesthesia differs from propofol anesthesia in the live rodent brain. *J Cereb Blood Flow Metab.* 2011; 31:1432–1442. [PubMed: 21266982]
33. Xu S, Ji Y, Chen X, Yang Y, Gullapalli R, Masri R. In vivo high-resolution localized (1)H MR spectroscopy in the awake rat brain at 7 T. *Magn Reson Med.* 2013; 69:937–943. [PubMed: 22570299]

Fig. 1.

(A) Slice-selective 2D absorptive-mode fly-back EPSI pulse sequence. The initial delay following the excitation pulse is set to enforce half-dwell ($\Delta/2$) or full-dwell (Δ) initial delays in the EPSI spectral dimension, $\delta = n\Delta/2 - \tau_1/2$. During this delay unwinding gradients in the EPSI spectral dimension and additional phase encode gradients can be applied. (B) Timing diagram for the absorptive-mode fly-back EPSI pulse sequence. The t_1 and t_2 axes represent the EPSI spatial and spectral times. The diagonal dotted lines represent data acquisition. The τ_g time is the time required for fly-back gradients between EPSI loops. The initial spectral delay is $\delta = n\Delta/2 - \tau_1/2$ (left panel). The choice of the initial delay removes the evolution in t_1 from the spectral dimension (right panel), and results in a small spatial shift in the EPSI spatial dimension. The diagrams are shown to scale for $n = 1$ (implemented in this work as $n = 2$).

Fig. 2.

Simulation of absorptive-mode fly-back EPSI using a full dwell delay. Simulation was performed for a ^{13}C base frequency of 11.2 MHz. The spatial sweep width was 10 kHz. The spectral sweep width was 22 ppm, centered at 172 ppm. The EPSI spatial acquisition time was 2.5 ms with 100 μs dwell time. The gradient fly-back time was 0.5 ms providing a 333 Hz sweep width. Four objects (expected chemical shifts of pyruvate (P-171.1 ppm), alanine (A-176 ppm), bicarbonate (B-161.8 ppm), and lactate (L-183.2 ppm)) were simulated centered at position zero Hz, with a spatial object width of 2 kHz. The spectral linewidth was set to 5.6 Hz. Spectra were zero-filled to 2 the original size prior to processing. The top two panels show the spectra through position 0. The bottom panels are EPSI spectral-spatial plots. (A) Absorptive-mode reconstruction (right panels) results in improved spectral

resolution over magnitude-mode reconstruction (left panels). (B) Note the small frequency dependent spatial shift, most conspicuous by comparing the bicarbonate and lactate positions (lower left panel), accentuated by dashed line. This is correctable by interpolation with spectrally dependent spatial shifting. (C) The distortion caused by miss-setting of the unwind gradient resulting in shifting the center of the echo by 2 dwell times (left panels) can be corrected by time shifting in t_1 , found by maximum entropy minimization (right panels). (D) The distortion caused by miss-setting of the fly-back gradient resulting in successive shifting by 0.1 dwell times (left panels), can be partially corrected by time-shifting (right panels), however the correction amplitude needs to be known a priori.

Fig. 3. Implementation and testing on ^{13}C phantoms (left panel) demonstrates feasibility of absorptive-mode EPSI imaging (middle panel), as compared to a 2D CSI sequence (right panel). The EPSI and CSI spectral plots correspond to the grid on the ^1H GRE image (left panel). The smaller phantom contains urea and the larger phantom contains urea, acetate, and alanine.

Fig. 4. Time resolved $[1-^{13}\text{C}]$ Pyruvate metabolism in the mouse brain ($n = 3$). Dynamic imaging of $[1-^{13}\text{C}]$ pyruvate metabolism of the mouse brain was performed on a permanent magnet operating at 1.05 T using an absorptive-mode EPSI sequence. (A) The 1D imaging grid is superimposed on a sagittal ^1H MRI image. The brain contour is shown in blue. (B) Average spectra from brain and vessels/soft tissues are shown. (C) The maximum brain lactate signal occurred 18 ± 1 s post injection, which was delayed relative to the soft tissues, in which the maximum lactate signal occurred 13 ± 3 s post injection (C).

Fig. 5. Static 2D $[1-^{13}\text{C}]$ Pyruvate metabolism in the mouse brain ($n = 3$). Representative static 2D slice-selective imaging of $[1-^{13}\text{C}]$ pyruvate metabolism of the mouse brain performed on a 1.05 T permanent magnet. Imaging was initiated 25 s after the start of the injection to maximize lactate signal buildup found in the dynamic experiment. (A) Contours of the brain and soft tissues are superimposed on a ^1H MRI of the mouse brain. A ^{13}C urea phantom is located in the bottom left corner. The raw EPSI imaging grid is overlaid on the image. (B) Average brain and soft tissue spectra are shown from a slice-selective absorptive-mode EPSI sequence. The EPSI dimension is vertical. In (C) and (D) the pyruvate and lactate maps are overlaid on the ^1H MRI of the mouse brain. The brain demonstrates increased metabolism of pyruvate to lactate, for example over the cerebral cortex (white arrow).